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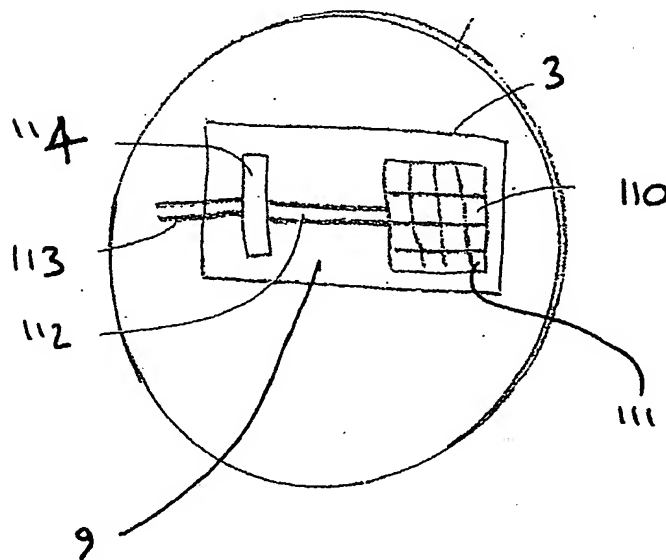
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(54) Title: **ELECTRODE**



(57) Abstract: There is described a solid state electrochemical sensor comprising a non-conducting substrate strip supporting a plurality of tracks, the tracks interconnecting connecting pins with at least a working electrode characterised in that it is fabricated on a silicon die. There is also described a method of determination of an analyte which comprises the use of such a solid state electrochemical sensor in conjunction with a discrete reference electrode.

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ELECTRODE

This invention relates to novel methods of analysis and to a novel form of electrode associated thereto.

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Conventional biosensor constructs of the type which may be used in a clinical biochemistry analyser comprise a platinum working electrode mounted with a membrane laminate, the precise nature of the laminate being varied depending upon, for example, the analyte. Generally, the laminate comprises an inner membrane, with selective properties, an outer diffusion limiting membrane and an enzyme layer sandwiched between the two membrane layers. The enzyme layer typically comprises an oxidase enzyme appropriate to the analyte to be measured. Thus, in use the analyte passes through the outer membrane, which may have a pore size around 30nm. The analyte then contacts the oxidase layer and reacts to generate hydrogen peroxide, the peroxide then passes through the selective membrane and is detected by the platinum electrode.

Such biosensors when incorporated in a laboratory analyser may provide precise and accurate results, but they suffer from a number of disadvantages including relatively high cost and high maintenance requirements. In addition, as the sensor is reused, it must be calibrated frequently. Oxidase type biosensors, as described above, have also suffered from the particular disadvantage that the inner selective membrane, designed to permit the flow of hydrogen peroxide, may also permit the flow of interferents e.g. paracetamol, giving rise to inaccurate measurements.

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In the 1980's, rapid test strips for measurement of glucose were further developed to enable electrochemical detection. Such biosensors may comprise a screen printed electrode track on an inert plastic substrate. Typically, the sensor itself comprises glucose oxidase enzyme incorporated in a carbon based working electrode, being covered by hydrophilic mesh. This type of biosensor provides some advantages over the laboratory based laminate. In particular, as it is disposable and provides only one

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test, it requires no ongoing calibration by the user, thereby reducing the running costs and complexity of test. Also, with devices of this type where hydrogen peroxide is replaced with an alternative electron transfer mediator, glucose can be detected using lower polarising voltages at the working electrode, thereby affording some improvement in selectivity. However, this type of biosensor suffers from a number of disadvantages. Due to the absence of a selective membrane, they cannot provide the degree of selectivity against interferents possible with the best selective membranes incorporated in a laminate type biosensors (ref. European Patent Application No. 0 225 094). In addition, due, *inter alia*, to the imprecision of the screen printing techniques often used in fabrication of the critical working electrode, this biosensor construct can only provide limited analytical precision. Thus, when configured as a disposable test, this biosensor provides inferior precision and accuracy against the conventional laminate system when incorporated in a laboratory analyser. In addition, it may be more vulnerable to matrix effects e.g. haematocrit.

Common to both laminate and strip type biosensor configurations is the stability problems associated with the use of an enzyme reagents. This has been addressed e.g. using crosslinking chemistries for laminate systems, and using carbon adsorption for strip sensors, however whilst these may relatively reduce loss of enzyme activity with time, stability *per se* will remain a constraint in products based on either of these approaches.

There has therefore long been a need for a sensor which is low cost, requires low maintenance and avoids the problems of enzyme stability and specificity, whilst providing the best possible accuracy and precision. Given the proven value of strip-format tests for rapid analysis, it would be preferable that a sensor with improved performance characteristics retain a strip format for ease of handling and use.

We have developed a novel sensor which overcomes the problems of prior art sensors by the use of silicon die technology used in tandem with an inert, plastic strip substrate. By the term silicon we mean silicon or a compound of silicon, for

example, silicon dioxide e.g. quartz. According to a first aspect of the invention we provide a solid state electrochemical sensor comprising a non-conducting substrate supporting a plurality of electrode tracks, and at least a working electrode fabricated on a silicon die.

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The substrate may additionally have a counter and/or reference electrode laid down on it. Alternatively, the reference and/or counter electrodes may be separate to the die based strip electrode. It may also have more multiple dies mounted on it and or multiple reference and/or counter electrodes. These may be individually addressable,
10 or may be addressed as one electrode or as combinations of electrodes.

In the solid state electrochemical sensor of the invention the non-conducting substrate strip may also support an analyte target electrode wherein the electrode tracks and the target electrode area are connected by an interconnect comprising a
15 globule of conducting material, for example, a silver globule.

The size of the silicon die may vary, thus the die may be from 100 to 500 μ m thick, preferably about 250 μ m thick, e.g. in the form of a silicon wafer. The bare die may have a size in the region of 1.1mm x 2mm, although the specific dimensions may be
20 varied.

In the solid state electrochemical sensors of the invention the non-conducting substrate preferably is both electrically and chemically inert. Such a substrate preferentially comprises a thin strip of material which may be e.g. a plastics material.
25 Such materials include, but are not limited to, polycarbonate, polyimide, polyvinylchloride, polyacetate. An especially preferred plastics material is PE (polyester).

At the proximal end of the inert substrate strip, the sensor of the invention may
30 advantageously be provided with one or more connector pins or pads, preferentially a plurality of connector pins or pads. Reference hereinafter to connector pins should

- be construed as including, optionally, connector pads. The connector pins may comprise any conventionally known conducting material, such as silver, gold, platinum, copper, etc. or any combination thereof. Preferred connector pins comprise a silver/resin system or a silver loaded ink, of the type used for screen printing inks.
- 5 The connector pins are integrated with the sensor trackwork which is used to connect up various components, e.g. the working and/or , reference and/or counter electrode(s) together if required with an identification resistor.

- The sensor may be connected to the target instrumentation via the connector pins,
- 10 either by directly inserting the sensor into a suitable sensor connector in the instrument casework, or by inserting the sensor into a sensor connector mounted in a separate probe, umbilically linked to the required instrumentation. Suitable instrumentation may be designed for handheld use or for benchtop operation.
- 15 The identification resistor is preferably a resistor device coated on the substrate which can be connected via the sensor connecting pins. Thus by changing the value of the resistor, it is possible to identify each different type of sensor by a specific resistance value or resistance range. Hence simply by inserting the sensor into the relevant connector and so attach the sensor to accompanying instrumentation, it is
- 20 possible for the instrumentation to determine the type of sensor and interrogate the sensor and process resulting data in a way specific to that sensor type, this includes selection of the required calibration. This has particular value where a menu of tests is required using a common instrumentation platform. Thus the act of attaching the sensor to the associated instrumentation is all that is required to configure the
- 25 instrumentation, greatly simplifying the use of the device and minimising operator error by insertion of the wrong sensor.

- The identification resistor can confirm the interrogation regime required to initialise the sensor, specific to the sensor type. Furthermore, it may determine the specific
- 30 instrument check sequence required. Addition of sample to the sensor can be

detected automatically by deviation of the initialising baseline beyond normal limits
e.g. +/- 3 standard deviations.

Furthermore, accidental reuse of a single use sensor can be obviated by tracking
5 baseline characteristics when initialising the sensor. In addition, further safeguarding
may be possible by employing an electrical type fuse incorporated either in the
substrate electrode tracking on the die fabricated trackwork connecting the die
fabricated electrode.

10 The sensor of the invention may be adapted disposable use, re-use or continuous use.

The trackwork is then typically coated with an insulating compound so as to expose
on the proximal connector pins and distal electrodes and prevent fluid ingress to the
interconnecting trackwork. Any conventionally known insulating compound may be
15 used. However, a preferred insulating compound is a resin, for example, an epoxy
resin, such as a UV curable epoxy resin.

The working or counter electrodes may comprise any electrically conductive material
adhered to a portion of the substrate. Thus, such an electrode may comprise silver,
20 copper, gold or platinum, or any combination thereof. An especially preferred
electrode is a silver or silver/silver chloride reference electrode and/or a silver
counter electrode.

Another novel aspect of the invention is the connection between the die fabricated
25 working electrode and the electrode trackwork. Such connections are conventionally
wire bond connection. However we have found a novel interconnect and a novel
method of interconnection.

According to this aspect of the invention we provide a sensor as hereinbefore
30 described characterised in that the die based working electrode is connected to the
electrode tracks by a globule of conducting material, known as a "glob top".

Preferentially, the conducting material of the interconnect is a metal, such as silver or gold, however silver is especially preferred to achieve high conductivity whilst minimising cost. The metal is preferentially deposited in a metal/resin/solvent based ink system suitable for screen printing, e.g. a silver loaded ink. Alternatively, it may be deposited by electroplating or vapour phase sputtering. Thus a silver interconnect may be made by applying a silver loaded ink between a connection pad and a conducting leg and sealed using a globtop as hereinbefore described, dispensed over the area.

10

Since a silver interconnect as hereinbefore described would be reactive if exposed to the solution being detected, it is an important aspect of the invention that the silver interconnect is encapsulated by an inert, dielectric coating. A variety of materials may be suitable, but it is preferable that the material be UV or thermally curable, is non-porous and is inert to the analyte and/or analyte solution. The adhesive used to fix the silicon die should also be non-porous, particularly since the adhesive must also act to prevent ingress of solvents and analytes to the silver interconnect. Dielectric materials need to be water impermeable and stable when exposed to water.

15

The silver globule is preferentially applied as a liquid, for example, as silver flakes in a polymer/solvent carrier system.

20

The die based electrode may comprise an array of electrodes or a single electrode. The electrode region of the die may comprise any conventionally known electrode materials e.g. platinum, palladium, gold or silver. However, preferentially, the electrode area comprises an array of electrodes preferentially an array of platinum electrodes. The array may be varied depending upon, inter alia, the analyte and also the silicon die and/or processing requirements. In a particularly preferred embodiment, the platinum electrode array comprises an arrangement of 4 x 4 electrodes.

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The silicon die is preferentially fixed to the substrate by a region of (die attach) adhesive. Any conventionally known adhesives may be used. However, it is important that the adhesive is resistant to both solvents and analytes which may be relevant to the steps required for further processing or the analytical use of the sensor.

5 Thus, commonly, for example, the adhesive must be resistant to alcohols, such as ethanol.

Preferentially the die attach adhesive, interconnecting silver/resin system and the dielectric encapsulant should be capable of being auto dispensed, permitting

10 automated production of the biosensors of the invention.

It is another novel aspect of the present invention to provide a die based electrode and/or substrate and/or substrate based electrodes which are in intimate contact with a membrane, preferably a selective membrane.

15 Thus according to a further aspect of the invention we provide a solid state electrochemical sensor comprising a non-conducting substrate supporting a plurality of electrode tracks and an analyte target area characterised in that one or more electrodes are in intimate contact with a selective membrane.

20 The use of a membrane may be advantageous with any conventionally known strip biosensor, particularly where the membrane is selective, and can minimise sample matrix effects. However, in an especially preferred biosensor, the invention comprises a biosensor provided with a silicon die working electrode as hereinbefore

25 described wherein at least the working electrode is in intimate contact with a hydrophilic, selective membrane.

It is preferred that substantially the whole of the biosensor construct which will be exposed to directly test sample is in intimate contact with the membrane. However,

30 it is within the scope of the present invention that only a portion, for example, the working electrode is in intimate contact with the membrane.

Thus the selective membrane is preferentially a permeable or a semi-permeable membrane. Hereinafter, such a membrane shall be described as a semi-permeable membrane.

5

The preferred semi-permeable membrane is one which is selective only to the indicator of choice. This may be the analyte itself if it is directly suitably electrochemically active, or it may be an indicator produced by a suitable detection chemistry. Detection chemistries may yield an indicator which may for example be a reaction product or co-substrate or co-factor generated by reaction of the analyte with an enzyme, or direct or indirect detection the reaction of analyte with and antibody or other affinity molecule. Suitable membranes may include those mentioned in various of our earlier Patents, including, *inter alia*, European Patent Applications No. 92906395.6, 93917908.1, 95906425.4, 96938307.4 and International Patent application No. PCT/GB97/03005.

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One especially preferred semi-permeable membrane may be that described in European Patent Application No. 0 225 094. Such a semi-permeable membrane may be provided with hydrophilic groups and/or groups which have an affinity to polar molecules. Such groups include, for example, sulphonic acid groups. Preferentially, the semi-permeable membrane may be formed from a material which comprises a sulphonated or unsulphonated polyarylsulphone or a sulphonated or unsulphonated polyarylketone as defined in European Patent Application No. 0 225 094. Most preferably the semi-permeable membrane is formed from a material which comprises a sulphonated polyarylsulphone or a sulphonated polyarylketone as defined in European Patent Application No. 0 225 094. The most preferred membrane is one which is formed from a material comprising a sulphonated polyarylsulphone

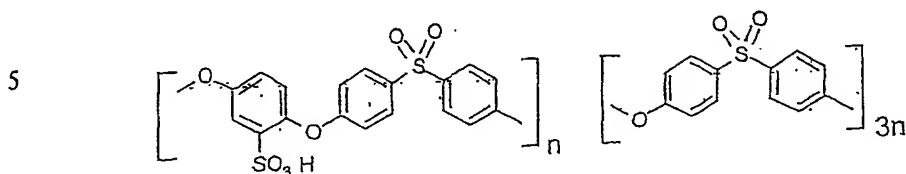
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A preferred material is a sulphonated polyarylsulphone of the type sulphonated polyether ether sulphone co-polymerised with polyether sulphone (SPEES/PES

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where the preferred sulphonation ratio is in the range 5-8. Such membrane materials, and are characterised by the general formula;



10 The membrane materials described in European Patent Application No. 0 225 094 are known to be especially useful in glucose determination as a selective barrier membrane permeable to hydrogen peroxide. However, we have surprisingly found that such membrane materials prevent the solid state electrochemical sensor of the invention from being attacked by alcohols, such as ethanol. The same advantage may
 15 be found when the sensor construct is exposed to other solvents, either for cleaning or further processing of the electrode. In these cases, the protective membrane may either reduce the amount of solvent able to access the electrode construct or, alternatively, it may entrap components which may subsequently leach so preventing these from interfering with electrochemical detection at the electrode surface.
 20 Materials which may be protected in this way with the construct cited here include dielectric coatings for insulation of tracking and interconnects, and die adhesive for mounting of dies on the required substrate.

If the reference and/or counter electrodes are deposited on the base substrate, these
 25 too may benefit from membrane coating, particularly the reference electrode. The working electrode may also, optionally, be membrane coated. In this case with the reference electrode, the electrode surface/solution interface is provided with a measure of control via the permselective membrane which is not available if the reference electrode surface is directly exposed to sample. In addition, membrane
 30 encapsulation of an electrolyte layer adjacent to the reference electrode is possible

which may enable further improvement in the control of the reference electrode voltage stability on exposure to samples.

5 Liquid sample may be applied to the sensor by simply immersing the sensor in the solution to be analysed together with any addition reference and/or counter electrodes and temperature sensor. Alternatively, it may be appropriate to add the sample to the sensor in a drop form or using lateral flow membrane techniques, wicks or sample absorbent pads. The use of a hydrophilic mesh sample dispersent may be advantageous, alternative sample delivery may be provided using a capillary fill
10 system or derivative of this approach.

Sample may be pretreated prior to application to the sensor (cite Bettix patent). This may be necessary for example to enable filtration, degassing or oxygenation. In addition, it may be desirable to perform modification of the sample e.g. by buffering,
15 addition of reagents or indicators, or addition of e.g. enzyme or antibody indicator chemistries.

The sensor may be interrogated by any appropriate electrochemical technique. This may include amperometry, voltammetry (including pulse based interrogation techniques), potentiometry or conductimetric techniques. Electrode construct can be
20 varied accordingly e.g. provision of counter electrode as well as a reference electrode in combination with the die based working electrode should a three electrode cell be required, alternatively provision of simply a combined counter/reference (pseudo reference) if this simplification is advantageous.

25 Sensors may be used to detect multiple analytes by varying the interrogation technique using the same sensor construct, and selecting the interrogation method required via the identification resistor. Alternatively, multiple die fabricated electrode sensors may be incorporated onto a single substrate strip. These may be
30 configured to detect the same analyte or different analytes.

Sensors constructed on the above type constructs may require correction of the resultant signals on sensor interrogation to allow for bias in the signals associated with batch to batch variation of sensor output for production sensors. This can be provided for using a sensor bias code to enable correction of the calibration intercept, the calibration slope, and any nonlinearity in the calibration. In addition correction for temperature sensitivity of the sensors can be made.

The semi-permeable membrane is preferentially permeable to the analyte, or a relevant analyte indicator, and impermeable or only partially permeable to other contaminants which may be present in the sample to be analysed. Thus, a variety of semi-permeable membranes may be used depending upon, *inter alia*, the nature of the analyte. Therefore, according a yet further feature of the invention we especially provide an alcohol, e.g. ethanol, sensor which includes semi-permeable membrane formed from a material comprising a sulphonated or unsulphonated polyarylsulphone or a sulphonated or unsulphonated polyarylketone as hereinbefore described.

We have found that coating the die mounted strip electrode with this type of membrane and preferably SPEES/PES, $s = 8$ produces a sensor which is surprisingly sensitive to detection of alcohols, and particularly ethanol. Ethanol can be detected at the platinum electrode with an enzyme (e.g. alcohol oxidase) acting to produce hydrogen peroxide which can be readily detected by electrochemistry. Alternatively, it is possible to eliminate the requirement for the enzyme by direct electrochemical detection of ethanol.

Ethanol is electrochemically reactive but requires relatively high polarising voltages and as the reaction product (aldehyde) also passivates the electrode, a more sophisticated interrogation than simple amperometry is required to achieve sensitive detection. We have found that on a bare electrode, ethanol can be detected using a 3 pulse pulsed amperometric detection (PAD) regime. A suitable typical pulse regime is 0.65V for 0.7s, 1.1V for 0.3s, -0.4V for 0.6s vs a silver/silver chloride reference electrode. However, with a SPEES/PES membrane coated electrode we have found a

two pulse regime to be optimal, using a regime of the type 1.4V for 0.3s, -0.4V for 0.2s vs silver/silver chloride. It is important to note that the high measurement voltage used at the membrane coated electrode (1.4V) is significantly greater than the voltage which can be applied to a bare electrode. The sensitivity enhancement by coating the electrode with SPEES/PES is of the order 4-5X greater than the bare electrode. Therefore we have found that contrary to membrane coated electrodes known elsewhere (original SPEES/PES patent) where increasing membrane thickness reduces permeability of the membrane and therefore sensitivity of the sensor, with the above alcohol sensor sensitivity increases with membrane thickness and as found previously, increases membrane thickness increases selectivity. Typical membrane thickness which we have found useful are in the range 2-10 μm .

Of note, the hydrophilic character of the SPEES/PES membrane facilitates rapid wet-up of the sensor from a dry-state storage condition. Therefore, sensors fabricated using this membrane have a rapid response time with steady state currents achievable within 30s. We have also found that as no enzyme is required with this sensor, it has very good stability characteristics after fabrication. We have found no detectable shift in sensor calibration after more than 2 months storage of these sensors.

- Having developed a SPEES/PES coated die mounted electrode, this provides a platform for development of biosensors, similar in construction to successful laminate type systems. In this case, the inner selective membrane is provided by SPEES/PES which is a highly selective membrane, particularly valuable for screening out interferents in clinical type samples (e.g. blood, serum, plasma).
- Whereas in conventional laminate systems, the membrane has to be cast and then manipulated in laminate fabrication as a pre-formed membrane, direct coating on the die mounted electrode provides a particularly convenient method of membrane fabrication avoiding handling of the delicate membrane post fabrication.
- Direct coating of the die mounted electrode strip ensures intimate contact of membrane with the electrode surface which superior is to pre-formed laminate type

biosensors where contact between the inner membrane and the electrode surface is enabled typically by mechanically clamping the laminate in place.

5 Enzyme type biosensors can be developed using a range of potential outer membranes (see earlier list) to provide diffusion limitation of substrate, protection from cells, colloids etc, encapsulation of the enzyme layer together with required chemistries/indicator systems. A range of enzymes can be employed which can be oxidases e.g. glucose, pyruvate, lactate, alcohol or dehydrogenases e.g. malate, alcohol, glucose. Thus it is possible to provide for encapsulation of enzymes,
10 reagents, cofactors, cosubstrates or other agents required to modify the sample e.g. buffer salts.

This sensor type can be used to detect immunoassay, for example in the type of format previously described (plasticised PVC patent, or immunoassay patent). It may
15 also be applicable to other ligand binding type detection systems.

The die mounted strip sensor may have reference and counter electrodes incorporated on the strip. These may be fabricated on the die itself, using the preferred materials described above, thus the strip provides the addition requirements – trackwork for
20 connecting electrodes, connector pins together with an identification resistor as may required. Alternatively, the die may only provide the working electrode or electrode array with the counter and reference electrodes be provided via fabrication on the strip. Combinations of the above two formats are possible. Alternatively, in a third format, reference and/or counter electrode are provided by additional electrodes
25 which may be presented to the sample in conjunction with the die mounted strip electrode.

An especially preferable configuration to maximise analytical accuracy and precision is the use of a die mounted strip electrode to provide a die fabricated working
30 electrode, a substrate fabricated counter electrode and a separate reference electrode presented to the sample in conjunction with the die mounted strip electrode. This

configuration may provide for the best possible reference electrode voltage accuracy and stability by enabling the use of a silver/silver chloride reference electrode in the presence of a suitable filling solution (e.g. silver chloride saturated 3M potassium chloride) protected from the sample using a ceramic frit (i.e. a conventional format
5 reference electrode with liquid junction and filling solution. Alternatively, other conventional reference electrode materials can be employed e.g. calomel in combination with other conventional reference electrode configurations and structures.

10 An additional feature to maximise the precision and accuracy of measurements made with the die mounted strip electrode is the use of a temperature sensor. This has value as electrochemical reactions measured at the die-based working electrode are temperature dependent. Thus precision and accuracy of measurements is improved by compensating the sensor responses for temperature changes. This has particular
15 value for measurement of samples which may be presented at a wide variation of temperatures e.g. in the measurement of wine or beer where sample temperature may range between 4°C - 30°C.

The temperature sensor may be mounted on the die mounted strip electrode by
20 fabricating on the electrode die itself, alternatively a temperature sensing device may be attached to the strip electrode in combination with the electrode die. The temperature sensor may alternatively be presented as a device separate from the die mounted strip electrode and presented to the sample in conjunction with the die mounted strip electrode together with any additional counter and/or reference
25 electrodes required.

Thus, according to a further aspect of the invention we provide a method of determination of an analyte. According to one aspect of the invention we provide a method of determination of an analyte which comprises the use of a solid state
30 electrochemical sensor in conjunction with a discrete reference electrode, said sensor comprising a non-conducting substrate supporting a plurality of tracks, the tracks

providing interconnection to an identification resistor, a working electrode and a counter electrode. The working electrode being fabricated on a silicone chip.

5 According to a further aspect of the invention we provide a method of determination of an analyte which comprises the use of a solid state electrochemical sensor comprising a non-conducting substrate supporting a plurality of tracks, the tracks interconnecting an identification electrode, a counter electrode and a die fabricated working electrode characterised in that it is in intimate contact with a selective membrane.

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In the method of the invention, the reference electrode may be integral to the solid state electrochemical sensor or alternatively it may comprise the use of a separate discrete reference electrode.

15 The method of the invention may be applied to the determination of a variety of analytes, however, specific analytes which may be mentioned are alcohols, such as ethanol and polysaccharides, such as glucose. According to one aspect of the invention we provide a method as hereinbefore described in which the analyte is glucose. According to a preferred aspect of the invention we provide a method as
20 hereinbefore described in which the analyte is ethanol.

In the method of the invention any conventionally known reference electrode may be used, indeed, this may vary depending, *inter alia*, upon the nature of the analyte. However, for use, for example, in alcohol determination the external reference
25 electrode may advantageously be a silver/silver chloride electrode immersed in a solution of 3M potassium chloride saturated with silver chloride and separated from the sample by means of a ceramic frit.

Since solid state electrochemical sensors conventionally comprise an integral
30 reference electrode, the solid state electrochemical sensor used in the method of the invention as hereinbefore described is therefore novel *per se*.

According to a further feature of the method of preparing a novel solid state sensor of the invention which comprises;

- 5 (i) printing a plurality of electrode tracks onto a non-conducting substrate;
- (ii) mounting a silicon die with an analyte target at one end of the non-conducting substrate support;
- 10 (iii) connecting the tracks to the silicon die with an interconnect region;
- (iv) coating the interconnect with an inert resin; and
- (v) coating the sensor with a selective membrane.

15

The aforementioned sequence of steps are preferentially carried out on a bare substrate material.

The process of the invention may include dicing and mounting the die onto a surf
20 tape using machines which are conventionally known *per se*. Thus, the bare die may be bonded onto a, e.g. screen printed substrate, using a screen printed adhesive, and an automatic pick and place machine.

The electrodes deposited on the base substrate can be prepared a number of
25 techniques including screen printing of the electrode onto the substrate, plating electrodes or deposition of electrodes by sputtering of metals from a vapour phase. The preferred technique used herein is screen printing which ability to produce the base substrate reference, counter and/or working electrodes with requisite tracking and identification resistor at low cost in high volume.

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With the die fabricated working electrode, preferentially the process of the invention comprises applying the electrode to the die by a sputtering technique. According to the process of the invention platinum sputtering is especially preferred when using photolithographic mask and etch techniques.

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According to a further aspect of the method of the invention, the method also comprises encapsulating the working electrode(s) and substrate deposited counter and/or reference electrodes in a selective membrane.

- 10 It is also a novel aspect of the invention to use selective membranes as hereinbefore described with a solid state electrochemical sensor as hereinbefore described.

Thus, according to a yet further aspect of the invention we provide the use of a membrane formed from a sulphonated or unsulphonated polyarylsulphone or a
15 sulphonated or unsulphonated polyarylketone, as defined in European Patent Application No. 0 225 094, in the manufacture of a solid state electrochemical sensor as hereinbefore described.

- 20 We especially provide the use wherein the sensor as hereinbefore described as an alcohol, e.g. ethanol, sensor.

As previously described, it is a particularly advantageous aspect of the present invention that the solid state electrochemical sensor can be utilised with a variety of analytes. Therefore, it is advantageous that identification means be included so any
25 related software can identify the nature of the analyte.

Thus according to a yet further aspect of the invention we provide a solid state sensor which is provided with identification means.

- 30 The identification means is preferentially a register, generally referred to as an ID resistor.

According to a further feature of the invention we provide a kit comprising at least a disposable solid state sensor and optionally a separate reference electrode.

- 5 The invention will now be described, by way of example only and with reference to the accompanying drawings, in which;
- Figure 1 shows the front, back and top views of the hand held instrument;
- Figure 2 shows a sensor of the invention;
- Figure 2a is a schematic representation of a plan view of the working electrode area
- 10 of the solid state electrochemical sensor of Figure 2;
- Figure 3 illustrates a reference electrode;
- Figure 4 illustrates a sample bottle;
- Figure 5 illustrates inserting a sleeve into the bottle of Figure 4;
- Figure 6 illustrates inserting the sensor into the sensor contact slot; and
- 15 Figure 7 illustrates the probe.

Referring to Figures 2 and 2a, a sensor (1) comprises an inert substrate strip (2) provided with an analyte target area (3), a working electrode (4) and counter electrode (5). Further electrodes (6) and (7) are connected to an identification

20 resistor (8). The analyte target area (3) comprises a silicon wafer (9) mounted on the strip and provided with platinum electrodes (10). The target area (3) comprises a silicon chip die (9), upon which is mounted an electrode area (110) comprising a plurality of, e.g. platinum electrodes (111) attached via a platinum connector (112) to a platinum terminal (113). The terminal (113) is connected via a silver interconnect

25 (114) to the electrode tracks (6 and 7).

The silicon chip die (9) is fixed to the surface of the strip (2) by the use of an adhesive layer (not shown). The adhesive layer fixes the surface of the silicon chip die (9) to the strip (2).

The substrate (2), the electrode tracks (6 and 7) and the target area (3) are coated with a selective membrane, e.g. a SPEES/PES membrane.

5 Referring to Figure 3, a reference electrode (11) comprises a temperature probe (12) and an electrode (13) in an electrode support (14). The support (14) is provided with an annular 'O'-ring (15).

Figures 4 and 5 illustrate a bottle as described in International Patent Application No. PCT/GB99/01820.

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Referring to Figure 6, a probe (16) is fitted with a reference electrode (13) and a temperature probe (12). The electrode support (14) is provided with a sensor contact slot (17) into which is inserted a sensor (1).

15 Referring to Figure 7, the sensor system is preferentially stored in the bottle of Figure 4, such that the reference electrode (13) and temperature probe (12) are placed in a storage solution (18).

20 The sensor may, preferentially, be provided with a protective film such as is described in our co-pending UK Patent application No. 011388.1

The use of the electrodes of the invention will now be described. Generally, the electrodes of the invention are intended to be used with, for example, the hand held unit that connects to the sensor probe as illustrated by Figure 1. The system can be
25 used to prompt the user to the next action by instructions displayed on and LCD.

An external reference can be prepared by gently squeezing the end of the teat and pulling away from the body of the electrode. The teat has a small amount of storage solution in it to keep the tip wet. Insert the reference electrode into the circular
30 socket in the probe and blot dry before use.

The docking station in the stabilising base is then filled with the reference storage solution to a depth of 10mm. The instrument should be calibrated. There are three levels of calibration available for the system. It is possible to improve the accuracy of the instrument by calibrating to the type of, for example, wine or beer to be analysed. To use the calibration function, samples with a known alcohol content must be available. The calibration levels are:

Level 1 is the default calibration supplied by the factory. The instrument has been calibrated using a wide range of different wines and beers and the default method should be adequate for most routine procedures.

Level 2, is the two-point calibration, which increases the accuracy of the system and analyses one sample at two different operating temperatures. This calibration will result in the most accurate alcohol measurement around the sample alcohol concentration used for calibration.

Level 3, the four-point calibration, analyses two samples with different alcohol content at two temperatures. This calibration will generate the most accurate results across the full alcohol concentration range.

For calibration sample preparation pasteurised samples that have been tested for alcohol by a reference method such as Gas Chromatography are required. The system requires warm and cold samples in order to provide the best accuracy over the operating temperature range. It is recommended that the samples are 5°C-10°C either side of the normal testing temperature at which the instrument will used.

To prepare a sample the caps from both chambers of the clean, dry sample bottle are removed and approximately 70 ml of the sample poured into the larger lower chamber (A). The caps are then firmly replaced on both chambers. Any remaining froth will not cause a problem. The sample bottle should be shaken vigorously and

the lower chamber cap removed to release the pressure and this should be repeated until no more gas is released.

5 Very gaseous samples, such as sparkling white wine, may require the process to be repeated more times.

The sample should be allowed to settle for 20 seconds. Meanwhile, the probe is placed into the docking station – the probe should be stored in this way when not in use, or in between samples to ensure that the sensor contact slot is protected and
10 remains dry. Refer to section titled Storing the system when not in use.

Remove the upper chamber cap and gently squeeze the bottle at the base of the lower chamber (A) to dispense sample into the upper chamber (B), ensuring that the chamber is completely filled. The sleeve is inserted into the top of the upper
15 chamber (B), pressing it firmly into place.

A sensor can be loaded into the contact slot on the probe (taking care not to push too hard). The sensor is inserted with the sensor side facing the reference electrode and temperature probe (see Fig. 6).

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The storage solution in the docking station should be changed approximately every 100 samples or more frequently if required.

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CLAIMS

1. A solid state electrochemical sensor comprising a non-conducting substrate strip supporting a plurality of tracks, the tracks interconnecting connecting pins with
5 at least a working electrode characterised in that it is fabricated on a silicon die.
2. A solid state electrochemical sensor according to Claim 1 characterised in that non-conducting substrate strip also supports an analyte target electrode wherein the electrode tracks and the target electrode area are connected by an interconnect
10 comprising a globule of conducting material.
3. A solid state electrochemical sensor according to Claim 2 characterised in that the interconnect comprises a silver globule.
- 15 4. A solid state electrochemical sensor according to Claim 1 characterised in that the silicon die material is selected from silicon and quartz.
5. A solid state electrochemical sensor according to Claim 1 characterised in that the silicon die material is silicon.
20
6. A solid state electrochemical sensor according to Claim 1 characterised in that the working electrode in the target area comprises an array of electrodes.
7. A solid state electrochemical sensor according to Claim 6 characterised in
25 that the array of electrodes is an array of platinum electrodes.
8. A solid state electrochemical sensor according to Claim 6 characterised in that the array of electrodes comprises an arrangement of 4 x 4 electrodes.

9. A solid state electrochemical sensor according to Claim 1 characterised in that the silicon chip target area support is fixed to the substrate by a region of adhesive.
- 5 10. A solid state electrochemical sensor according to Claim 9 characterised in that the adhesive is resistant to both solvents and analytes for which the sensor may be utilised.
11. A solid state electrochemical sensor according to Claim 9 characterised in
10 that the adhesive is resistant to ethanol.
12. A solid state electrochemical sensor according to Claim 1 characterised in that the electrode tracks comprise an identification electrode, a working electrode and a reference electrode.
- 15 13. A solid state electrochemical sensor comprising a non-conducting substrate supporting a plurality of electrode tracks, the electrode tracks comprising at least an identification electrode and a working electrode characterised in that at least the target area of the sensor is in intimate contact with a selective membrane.
- 20 14. A solid state electrochemical sensor according to Claim 13 characterised in that the sensor is a solid state electrochemical sensor comprising a non-conducting substrate supporting a plurality of electrode tracks, the electrode tracks comprising at least an identification electrode and a working electrode wherein the sensor has an
25 analyte target area which comprises a target electrode mounted on a silicone chip.
15. A solid state electrochemical sensor according to Claim 13 characterised in that substantially the whole of the sensor is in intimate contact with a selective membrane.

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16. A solid state electrochemical sensor according to Claim 13 characterised in that the membrane is readily wettable.
17. A solid state electrochemical sensor according to Claim 13 characterised in that the membrane is formed from a material described in European Patent Application No. 0 225 094.
18. A solid state electrochemical sensor according to Claim 17 characterised in that the membrane is formed from a sulphonated or unsulphonated polyarylsulphone or a sulphonated or unsulphonated polyarylketone.
19. A solid state electrochemical sensor according to Claim 18 characterised in that the membrane is formed from a sulphonated polyarylsulphone.
20. A solid state electrochemical sensor according to Claim 19 characterised in that the membrane is a SPEES/PES membrane.
21. A solid state electrochemical sensor according to Claim 20 characterised in that the membrane is a SPEES/PES membrane with an "S" number of 8.
22. A solid state electrochemical sensor according to Claim 13 characterised in that the membrane comprises a plurality of layers
23. A solid state electrochemical sensor according to Claim 22 characterised in that the membrane comprises at least one layer which is an enzyme containing layer or an antibody containing layer.
24. A solid state electrochemical sensor according to Claims 1 or 13 characterised in that the substrate is formed from a material selected from cellulose acetate, a polyimide, a polycarbonate or a polyester.

25. A solid state electrochemical sensor according to Claim 24 characterised in that the substrate is formed from a polyester material.
26. A solid state electrochemical sensor according to Claim 24 characterised in that the substrate is formed from a polycarbonate material.
27. A solid state electrochemical sensor according to Claims 1 or 13 characterised in that at one end of the substrate strip, the sensor is provided with one or more contact pads.
28. A solid state electrochemical sensor according to Claim 27 characterised in that the connector pins comprise a silver plate on a copper base.
29. A solid state electrochemical sensor according to Claims 1 or 13 characterised in that the sensor is provided with an identification means.
30. A solid state electrochemical sensor according to Claim 29 characterised in that the identification means is a resistor.
31. A solid state electrochemical sensor comprising a non-conducting substrate supporting a plurality of electrode tracks and an analyte target electrode area characterised in that the electrode tracks and the target electrode area are connected by an interconnect comprising a globule of conducting material.
32. A solid state electrochemical sensor according to Claim 31 characterised in that the interconnect comprises a silver globule.
33. A solid state electrochemical sensor according to Claim 31 characterised in that the interconnect is surrounded by an inert cover.

34. A solid state electrochemical sensor according to Claim 33 characterised in that the inert cover is a resin.
35. A solid state electrochemical sensor according to Claim 34 characterised in
5 that the resin is UV or thermally curable.
36. A solid state electrochemical sensor according to Claim 34 characterised in that the resin is non-porous.
- 10 37. A solid state electrochemical sensor according to Claims 1 or 11 characterised in that the sensor is an ethanol sensor and the membrane is a SPEES/PES membrane with an "S" number of 8.
- 15 38. A method of determination of an analyte which comprises the use of a solid state electrochemical sensor in conjunction with a discrete reference electrode, said sensor comprising a non-conducting substrate supporting a plurality of electrode tracks, the electrode tracks comprising at least an identification electrode and a working electrode, the sensor having an analyte target area, which comprises a portion of the working electrode mounted on a silicon chip.
- 20 39. A method of determination of an analyte which comprises the use of a solid state electrochemical sensor comprising a non-conducting substrate supporting a plurality of electrode tracks, the electrode tracks comprising at least an identification electrode and a working electrode characterised in that at least the target area of the
25 sensor is in intimate contact with a selective membrane.
40. A method according to Claims 38 or 39 characterised in that the analyte is glucose.
- 30 41. A method according to Claims 38 or 39 characterised in that the analyte is ethanol.

42. A method according to Claims 38 or 39 characterised in that the sensor comprises an integral reference electrode.

5 43. A method according to Claims 38 or 39 characterised in that the sensor is provided with a separate reference electrode.

44. A method according to Claim 43 characterised in that the reference electrode is a silver/silver chloride electrode.

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45. A solid state electrochemical sensor comprising a non-conducting substrate supporting a plurality of electrode tracks, the electrode tracks comprising at least an identification electrode and a working electrode characterised in that the sensor has an analyte target area which comprises a target electrode mounted on a silicone chip.

15 at least a substantial portion of the target area of the sensor is in intimate contact with a selective membrane.

46. A method of forming a solid state electrochemical sensor according to Claim 45 which comprises;

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(i) printing a plurality of electrode tracks onto a non-conducting substrate;

(ii) mounting a silicon chip with an analyte target at one end of the non-conducting substrate support;

25

(iii) connecting the electrode to the silicon chip with an interconnect region;

(iv) coating the interconnect with an inert resin; and

30 (v) coating the sensor with a selective membrane.

47. A method according to Claim 46 characterised in that the printing is carried out by a sputtering process.

48. A method according to Claim 46 characterised in that the target area of the electrode is encased in a selective membrane.

49. A method according to Claim 48 characterised in that the size exclusion membrane is formed from a sulphonated or unsulphonated polyarylsulphone or a sulphonated or unsulphonated polyarylketone

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50. The use of a selective membrane in the manufacture of a solid state electrochemical sensor.

51. The use according to Claim 50 characterised in that the selective membrane is formed from SPEES/PES.

15

52. The use according to Claim 50 characterised in that the a solid state electrochemical sensor is an ethanol, sensor.

53. A kit comprising at least a solid state electrochemical sensor according to Claims 1 or 13 and optionally a separate reference electrode.

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54. A method or a sensor device substantially as described with reference to the accompanying examples.

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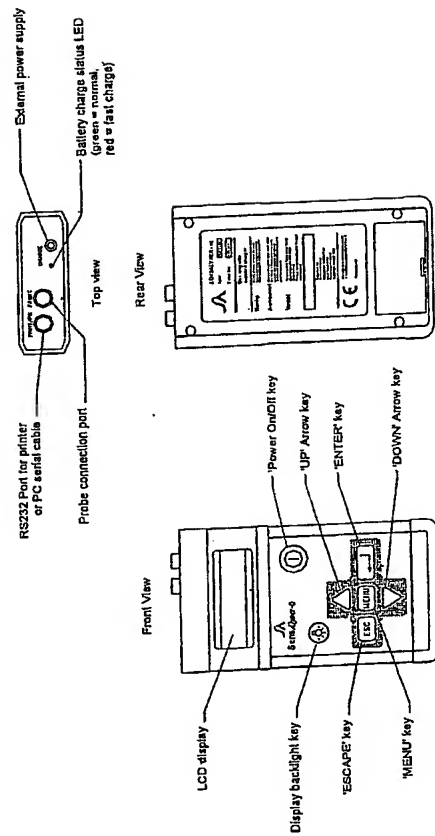


Fig. 1

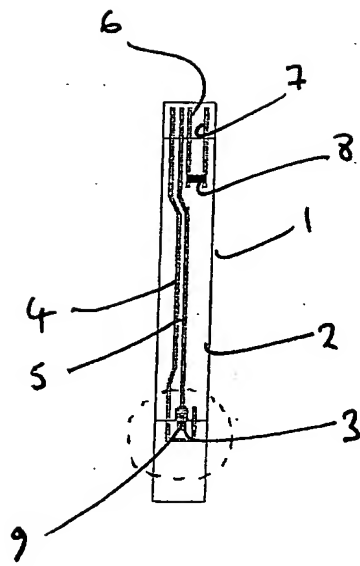


Fig. 2

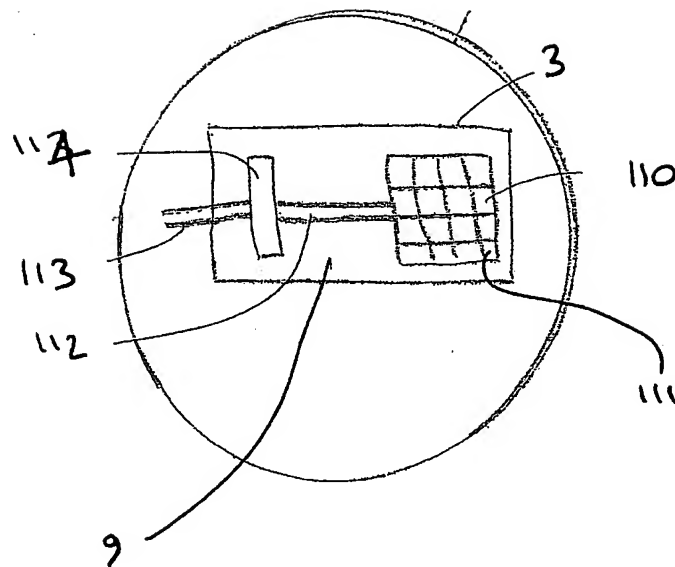


Fig. 2a

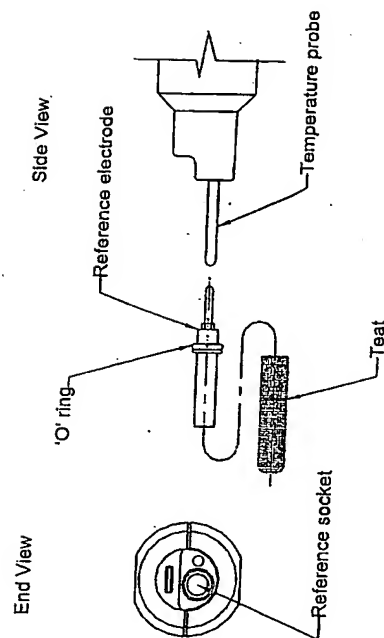


Fig. 3

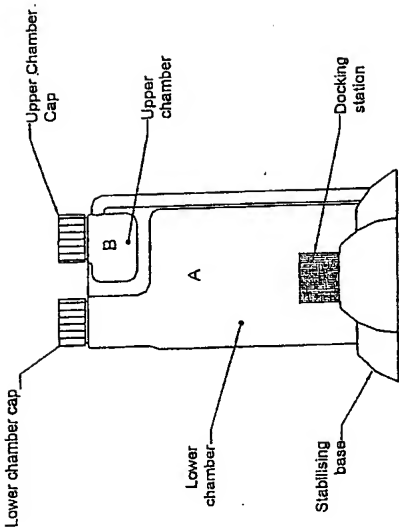


Fig. 4

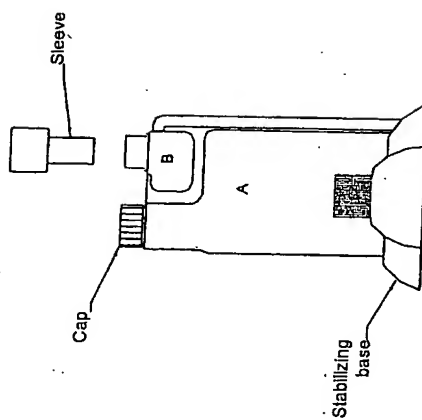


Fig. 5

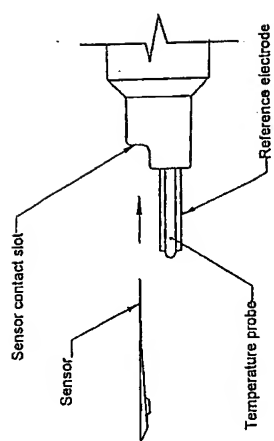


Fig. 6

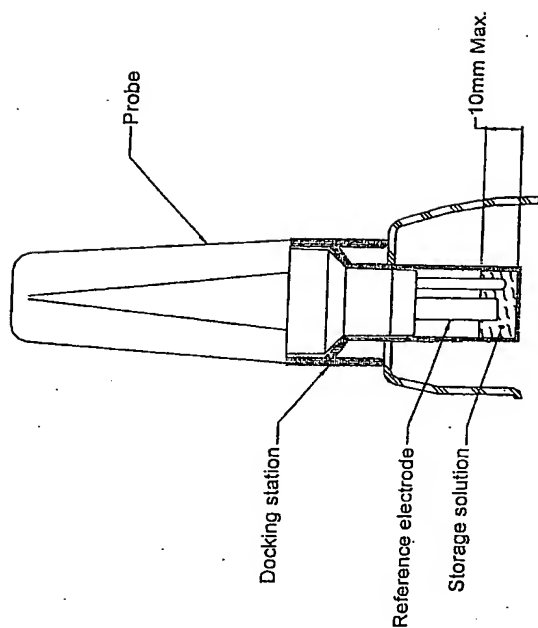


Fig. 7

